## III. REMARKS

### *Claim Status*

Claims 1-2, 6-13 and 18-34 are under current examination. Claims 1-2, 6-13 and 18-34 stand rejected. Claims 1, 18, 21 and 24 have been amended. Claims 2 and 27 have been cancelled.

### *Claim Rejections - 35 USC § 103*

Claims 1-2, and 10-13 and claims 1-2, 6-13 and 18-34 are rejected under 35 U.S.C. 103(a) as being obvious over Margolskee (US-5,817,759, issued 6 October 1998) in view of Yao et al. (US-7,041,457, issued 9 May 2006) and further in view of Ruiz-Avila et al. (PNAS. July 17 2001. vol.98; No.15: 8868-8873).

**a) Prosecution History**

Applicant and the examiner have reduced the outstanding issues to whether or not the references cited by the examiner show a high enough degree of predictability to render applicants instant claims obvious. Secondarily the issue of the degree of homology described in the art is at a level sufficient to allow one skilled in the art to produce applicant's claimed compositions with the expectation that the claimed compositions would function as successfully as they do for their intended purposes.

Accordingly, the existence of a *prima facie* case of obviousness therefore is contingent upon the correctness of the examiner's position on these two issues.

**b) Applicant's Claims**

Applicant discloses and claims a $G_{\alpha q\text{-}Gustducin}$ chimeric G-protein wherein the last 44 amino acids of the $G_{\alpha q}$ protein sequence are replaced with a 44 amino acid unit of Gustducin, G15/gust 44 and G16/gust44.

**b) Summary of Argument**

Applicant respectfully traverses the Examiner's rejection of the indicated claims in view of the Margolskee, Yao and Ruiz-Avila references.

For sake of brevity, applicant herein incorporates by reference the prior remarks made with regard to these references as being equally relevant to the current grounds of rejection against the claims.

To support obviousness rejection, the examiner relies Margolskee and Ruiz-Avila et al., "which indicate that the carboxy terminus is important to the function of sensation-specific G proteins." The examiner states that "Yao et al. suggests 'chimeric Gq variants and the isolated nucleic acids encoding the same. In one embodiment, the chimeric Gq protein variants comprise C-terminal sequences from transducin or $Gaq_{if}$.'"

With regard to homology, the examiner cites Margolskee "who teaches 'among mammals...the α subunits of gustducin and the transducins comprise a subfamily of closely related proteins'". Continuing, "Ruiz-Avila et al. seems to suggest both (1) the strong homology between gustducin and the transducins and (2) the importance of the C-terminus, …"

The examiner is unable to find art that couples gustducin to Gq protein variant and so relies on Yao et al. to teach "chimeric Gq protein variants [which] comprise C-terminal sequences from transducin...(col.3, lines 10-13)...[having]...up to 44 amino acids of the C terminus of transducin" (col.5, lines 16-19 and 22-23).

As the examiner has not been persuaded by applicants previous arguments, applicant submits herewith additional art independently demonstrating the lack of replicable functionality when even slight changes are made to the constituents of the chimeric protein.

Applicant previously stressed that no predictions are possible regarding whether specific chimeric embodiments are functional, [i.e. whether they would be promiscuous and transmit a signal to the receptor strong enough to be useful in a screening method for the embodiments claimed and for even the more similar ones not currently claimed.

To work in a screening method, binding and/or signal transduction activity are both necessary, but not sufficient, prerequisites.

Changes in the constituents of the chimera may affect the three dimensional shape and other required functionalities, including, in particular, promiscuity, which the present invention successfully increases. An increase in promiscuity of a given chimeric protein is at least as unpredictable as its general functionality. The the same principle also applies to signal strength.

Yao fails to demonstrate promiscuity even for the mouse Gαq-protein variants (MGq(DeltaN-HVD-HA)-t5 and MGq(DeltaN-HVD-

HA)-t44) he discloses: the only functional test in Yao is one using a mouse bitter receptor (MT2R5). It is therefore unclear from Yao whether the receptors will also work for sweet/umami.

The Examiner argues there was a low level of unpredictability, predictability being confirmed by homologies suggested in the prior art.

> "The applicant takes Ruiz-Avila as indicating unpredictability, but the examiner interprets this art as reinforcing the criticality of the C-terminus. Together with the suggestion of Yao to make chimeric G-proteins comprising the c-terminus of transducin, the teachings of Margolskee and Ruiz-Avila indicate similarities between transducin and gustducin and collectively suggest $G_{aq\text{-}Gustducin}$ chimeric G-protein comprising a substituted 44 amino acid carboxy terminus where such 44 amino acid unit of Gustducin is the last 44 amino acids of SEQ ID NO:2."
>
> The examiner concludes "There is really a low level of unpredictability, contrary to the applicant's assertion. The homologies suggested by the cited art confirm the predictability of the similar chimeric sense receptors."
>
> Applicant respectfully disagrees.

The above quoted portion of the office action is based on a modular concept that doesn't take into consideration the fact that biological entities are not mechanical "modules" that can be easily exchanged with similar ones and be expected to work in a different context.

The presence of any transducin-similar C-terminal sequence alone will not <u>necessarily</u> provide increased promiscuity and strong signal strength in combination with all specific Gαq

class proteins, for example, human Gαq (Yao used mouse Gαq), or other Gαq class proteins such as the presently claimed $G_{15}/G_{16}$.

Notably, the present claims are directed to a combination with $G_{15}/G_{16}$, which from Yao is known to give poor signal strength.

> "Similar problems arise when using $G_{\alpha 15/\alpha 16}$ to identify ligands of ORs and T2Rs (bitter taste receptors) in that (1) calcium responses to odorants are small and quickly desensitized for ORs in $G_{\alpha 15/\alpha 16}$ transiently transfected cells (Krautwurst et al., 1998); (2) most T2Rs remain orphan using cell lines stably transfected with Gα15 (Adler et al., 2000; Chandrashekar et al., 2000); and (3) threshold concentration of denatonium measured is at least one order higher than expected for bitter receptors, hT2R4 and mT2R8 expressed in cells stably transfected with Ga15 (Adler et al., 2000; Chandrashekar et al., 2000). These problems suggest that the coupling efficiency between ORs/T2Rs and $G_{\alpha 15/\alpha 16}$ is weak and may vary within the family of ORs and T2Rs."(Yao, col. 2, lines 37-50)

To highlight the correctness of applicant's position, and as further evidence of the art recognized level of unpredictability, applicant submits an additional reference supporting applicant's position: Takashi Ueda et al. 2003, The Journal of Neuroscience 23(19):7376-7380, published well after the priority date of the present application.

This publication is directed to various chimeric protein variants employing the $G_q$-class G-protein $G_{16}$:

- $G_{16gust5}$
- $G_{16gust11}$
- $G_{16gust23}$
- $G_{16/gust37}$
- $G_{16/gust44}$

Ueda et al. tested these domains not to increase signal strength in a screen, but to "determine a specific domain of gustducin necessary for T2R coupling", with a view to determining the molecular basis for the interaction, about which "little is known", according to Ueda.

Taking into account Ruiz-Avila, if the art indeed had a minimum level of predictability, the $G_{16gust5}$ variant, which according to the Examiner's position is obvious over the $MG_{\alpha q\text{-}transducin5}$ version of Yao in view of Margolskee and Ruiz-Avila, would be expected to work, and work similarly to the variants comprising a longer part of the gustducin C-terminus.

However, this is not the case: apparently the $G_{16gust5}$ variant does not work, or at least significantly differs from how the $G_{16gust44}$ variant works, as set forth in Ueda's abstract:

> "Bitter taste perception is a conserved chemical sense against the ingestion of poisonous substances in mammals. A multigene family of G-protein-coupled receptors, T2R (so-called TAS2R or TRB) receptors and a G-protein a subunit (Gα), gustducin, are believed to be key molecules for its perception, but little is known about the molecular basis for its interaction. Here, we use a heterologous expression system to determine a specific domain of gustducin necessary for T2R coupling. Two chimeric Ga16 proteins harboring 37 and 44 gustducin-specific sequences at their C termini (G16/gust37 and G16/gust44) responded to different T2R receptors with known ligands, but G16/gust 23, G16/gust11, and G16/gust5 did not." (Ueda, Abstract, lines 1-6)

Similarly, the G16/gust11 variant and the supposedly equally predictable G16/gust23 variant also do not work as well

as the G16/gust44 does. According to Ueda et al., both showed no signals while G16/gust44 showed high signals.

The Examiner's argument and basis for rejection of applicant's claims require predictability in this respect. To succeed is establishing a *prima facie* case of obviousness, all of these variants, which have a transducin C-terminus of 5 and up to 44 amino acids as taught by Yao, i.e. including the -t5 variant, would have to actually work giving robust signal strength in a screening method.

They did not.

On the contrary Ueda reports that none of these variants responded to different T2R receptors with known ligands; contrary to the position taken by the examiner, neither of the chimeric proteins having shorter C-termini worked, thereby clearly demonstrating a high level of unpredictability.

This is a direct and clear demonstration that while combining known module A with known module B1, and alternatively B2 may yield predictable similar results in other arts, this is not the case for complex biological systems where components A and B interact in unpredictable ways.

DNA sequences, when translated into proteins, form a complex three dimensional shape that interacts with other complex three dimensional shapes within a signaling cascade of many members, introducing yet another level of complexity. Small changes of either individual modules themselves or their three dimensional context in which that they are employed (e.g. combining a different C-terminal domain with a different protein backbone such as exchanging G16 for Gαq, or hG16 with mGαq as is

the case here) often has unpredictable results, as clearly demonstrated by Ueda et al.

Before trying it out experimentally, i.e. without applying hindsight, it is impossible to predict which domains will continue to bind, interact and transmit their signal with all interacting partners in their new three dimensional environment. A chimeric protein might lose or acquire new unwanted functionality leading to incompatibility with any one of its partners, either in binding, in interaction or in signal transmission.

For example, G-proteins are heterotrimeric and consist of alpha, beta and gamma subunits, so any change would be required not to significantly affect the interaction with the other subunits. Yao et al. is in agreement with this point, stressing the central part of this type of G-protein with various components in signaling:

> "Intracellular signaling is mediated through various effector enzymes, including cGMP phosphodiesterase, phospholipase C, adenylate cyclase, etc. (see Kinnamon & Margolskee, 1996, Curr. Opinion Neurobiol. 6: 506 513). Most effector proteins interact with the Gα, although Gβ γ subunits also contribute to the specificity of receptor-G protein coupling (Xu et al., 1998, J. Biol. Chem. 273(42): 27275 79)." (Yao, col. 1, lines 35-42)

Further, even if general functionality remained, it is even more unclear whether the chimeric protein would have the necessary signal strength for a screening method or the desired increased promiscuity, e.g. being activated and transmitting the signal of both bitter and sweet receptors.

Ueda et al. did not set out to establish a system of increased promiscuity and signal strength. Instead Ueda was researching the relative importance of the various domains, and appears rather surprised by the findings, especially by the fact that the G16/gust23 variant did not work, even though the a5 helix was believed to be the major factor:

> "In contrast, G16/gust23 that contained the $_{a}$5 helix of gustducin appeared not to associate, although numerous studies have attested to the importance of the α5 helix in receptor coupling. Similarly, G16/gust11 and G16/gust5 did not cause T2R activity. These results indicated that the $_{a}$5 helix and extreme C terminus of gustducin were insufficient for detection of T2R activities, and the β6 sheet, in addition to the α5 and C-terminal β-sheet, is indispensable for signal transduction of T2Rs." (Ueda 7379, top of right col.)

This indispensability was not previously known, Ueda being published after the priority date of the present application.

In view of Yao, Ueda's results may at first glance seem somewhat surprising, provided one assumes a predictability that the field simply does not have. The skilled person might hope and try out variations to see which one works, but would certainly not do so with a reasonable expectation of success. Notably, Yao exemplified and specifically disclosed only the mouse Gαq-t5 and mouse Gαq-t44 variants (but not hGαq-t5 nor hGαq-t44, nor any mouse or human G16 variant, compare table I in Yao).

Further, Yao tested only the mT2R5 receptor, thereby failing to show increased promiscuity.

The examiner asserts that "regar[d]less of the overall "low 58% homology" between gustducin and transducin, the portion

important for function (44 aminoacids of the carboxy terminus), there is a much greater homology."

However, the C-terminus, or any component, cannot be considered in isolation, and a high homology is not necessarily an indicator of predictability and likelihood of success.

The irrelevance of a high degree of homology and the high degree of unpredictability in the art is further demonstrated by one of the prior art documents cited by the Examiner, Ruiz-Avila, which is discussed in Ueda. A gustducin mutant that is identical but for the exchange of only one amino acid in the extreme C-terminus results in a loss of the ability to activate receptors, as shown in the discussion of Ruiz-Avila in Ueda, Ueda page 7380:

> "Indeed, a gustducin mutant containing a glycine-to-proline substitution at position −3 can bind to taste receptor Gβγ subunits and the effector, but it cannot be activated by receptors (Ruiz-Avila et al., 2001). Therefore, the extreme C terminus may also play an important role in transduction via the gustducin, Gαt1, Gαt2, and Gαi2 of T2R taste receptors." (Ueda, left col., lines 11-16)

This highlights the significance of the difference between transducin and gustducin (6 aminoacids) in the short C-terminal stretch of only 44 aminoacids in the claimed chimeric proteins.

These are combined with a different backbone, G16/G15, while Yao merely disclosed chimeric proteins comprising the two mouse variants of a different specific Gαq class protein, the one that gave the class its name, Gαq: MGq(DeltaN-HVD-HA)-t5 and MGq(DeltaN-HVD-HA)-t44 (both using transducin C-terminal sequences, with the five "t5" amino acids of transducin being identical to those of gustducin).

Applying the Examiner's modular view and dismissing potential interactions of C-terminal part with backbone, -t5 chimeric proteins (MGq(DeltaN-HVD-HA)-t5 and G16/gust5) being more similar to each other than the ones currently claimed, as at least the C-terminal part is identical, the difference being "only" in the combination with a different backbone, G16-t5 aka G16-gust5 would be predicted to work.

However, even if using the G16-t5 (=G16/gust5) chimeric variant identical in the "critical" C-terminal part, the results differ significantly, as Ueda shows for the G16-gust5 chimeric variant in comparison to the G16-t44 variant.

This further demonstrates that not even partial identity (t5/gust5) of a "critical" part is a good indicator of transferability, and success cannot be predicted.

Accordingly, the differing C-terminus of the presently claimed proteins (Yao's t44 C-terminus differs from the C-terminus of the claimed gust44 chimera in 6 of its 44 aminoacids, having a homology of 86.3% in the C-terminal part) is even less predictable in its interaction with the backbone, especially with the different backbone of G16/G15.

Ueda's results demonstrate that homology cannot be considered in "modules", and accordingly the total homology (which is very low, 57%, as previously explained) may be relevant as well and has to be considered in addition to the partial homology. Receptor proteins function in the three-dimensional context with their G-protein interaction partners.

When cutting out a specific "module" and transplanting it elsewhere, regardless of a higher degree of homology (or even identity, as shown by Ueda for the G16-t5/-gust5) in the

transplant, this does not mean it will work in its new surroundings, e.g. with a new G16/G15 backbone. In particular, it is not predictable whether the new variant would provide a signal strength useful for screening or increase promiscuity.

To illustrate, even if kidney A and kidney B are the identical organ "module", successful transplantation depends on minute characteristics and compatibility with the body the kidney is transplanted to. The "kidney" C-terminal module in our case is not even identical, and it is "transplanted" to a different backbone:

G16 or its ortholog G15, both share a low degree of homology of less than 57% similarity to Gαq, which, according to Yao, is a high divergence that should result in significant differences in efficiency and selectivity of receptor coupling:

> "Protein sequence similarity between Gαq and G α15/Gαq16 is less than 57% (FIG. 1). Accordingly, such high divergence should result in significant differences in efficiency and selectivity of receptor coupling. The identification of functionally active Gq protein variants could allow for the pharmacological and genetic modulation of sensory transduction pathways." (Yao, col. 4, lines 21-27)

Notably, Yao mentions G15/G16 only in general, enumerating proteins belonging to the same class as Gαq, and in particular points out the differences to Gαq ("Protein sequence similarity ... less than 57%", "high divergence"). Even for the variants disclosed (Gαq itself), no increased promiscuity is shown, as the variants are tested with only one receptor (compare Yao's table 1).

Furthermore, Yao points out problems with Gαq class proteins, and especially G16/G15, which are not true universal adaptors. In particular, signal strength is a problem:

> "Despite their promiscuity, however, Gαq class subunits do not mediate all GPCR--effector interactions. For instance, human Gα16 and its murine counterpart Gα5 are promiscuous G proteins in that they couple to GPCRs of different G protein families (Offermanns and Simon, 1995; Negulescu et al., 1997). However, they are not true universal adapters for GPCRs in that there are at least 11 GPCRs reported to be incapable of activating G.alpha.15/G.alpha.16 (Wu et al., 1992; Arai et al., 1996; Kuang et al., 1996; Lee et al., 1998; Parmentier et al., 1998; Mody et al., 2000). Similar problems arise when using Gα15/α16 to identify ligands of ORs and T2Rs (bitter taste receptors) in that (1) calcium responses to odorants are small and quickly desensitized for ORs in Gα15/α16 transiently transfected cells (Krautwurst et al., 1998); (2) most T2Rs remain orphan using cell lines stably transfected with Gα15 (Adler et al., 2000; Chandrashekar et al., 2000); and (3) threshold concentration of denatonium measured is at least one order higher than expected for bitter receptors, hT2R4 and mT2R8 expressed in cells stably transfected with Gα15 (Adler et al., 2000; Chandrashekar et al., 2000). These problems suggest that the coupling efficiency between ORs/T2Rs and Gα15/α16 is weak and may vary within the family of ORs and T2Rs." (Yao, col. 2, lines 28-50)

Furthermore the paralogs (G16/15 versus Gαq) are not very conserved, suggesting distinct functions according to Yao, even though some activities are similar:

> "Signaling specificity among α subunits of the same class having similar biochemical functions is not well understood in vivo. For instance, the Gαq (Gq) class includes four proteins expressed in mammals, called Gαq, Gα11, Gαq14, and Gαq15 (in mice, Gα16 in humans). Whereas orthologs of these subunits are highly

> conserved across species (99, 97, 96 and 85% identity, respectively), paralogs of these subunits (expressed in the same species) are not as conserved. This suggests that each type of subunit in the Gq class has a distinct function, however, when transfected into Sf9 cells, the subunits stimulated phospholipase C with similar potency and showed similar activities (Nakamura et al., 1995, J. Biol. Chem. 270: 6246 6253). Xu and colleagues subsequently showed by gene knockouts in mice that Gq.sub.60 subunits promiscuously couple to several different receptors in various cell types (1998, J. Biol. Chem. 273(42): 27275)." (col. 1, line 56 to col. 2, line 4)

This would appear to discourage the skilled artisan from replacing Gαq with G16/G15, or at least indicate unpredictability expecting different results, in particular if the C-terminal module is replaced as well.

In any case, it remains that the result is unpredictable, as shown by Ueda's G16gust5 variant which does not appear to work, and certainly works differently from G16gust44 which Ueda tested in parallel.

***Conclusion***

Since the existence of a *prima facie* case of obviousness therefore is contingent upon the correctness of the examiner's position 1) as to whether or not the references cited by the examiner show a high enough degree of predictability to render applicants instant claims obvious and 2) secondarily, whether the degree of homology described in the art is at a level sufficient to allow one skilled in the art to produce applicant's claimed compositions with the expectation that the claimed compositions would function as successfully as they do for their intended purposes and applicant has demonstrated by extrinsic factual

scientific evidence that neither predictability nor homology exist, the *prima facie* case upon which the rejection is based has been negated and

Accordingly, favorable reconsideration of and withdrawal of the current rejection of the present claims is solicited.

Should the Examiner in charge of this application believe that telephonic communication with the undersigned would meaningfully advance the prosecution of this application towards allowance, the Examiner is invited to contact the undersigned at his earliest convenience.

Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

Respectfully submitted,
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